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BIOCHEMICAL AND MORPHOLOGICAL DIFFERENTIATION IN SPANISH AND MOROCCAN POPULATIONS OF *DISCOGLOSSUS* AND THE DESCRIPTION OF A NEW SPECIES FROM SOUTHERN SPAIN (AMPHIBIA, ANURA, DISCOGLOSSIDAE)

STEPHEN D. BUSACK^{1,2}

Research Associate, Section of Amphibians and Reptiles

ABSTRACT

Biochemical and morphological divergence among Moroccan and Iberian populations suggests that populations of *Discoglossus* inhabiting these regions are not conspecific. Northern Moroccan *Discoglossus* are assigned to *D. pictus*; *Discoglossus galganoi* Capula et al. 1985 inhabits the Iberian peninsula to the north of the Guadalquivir River basin, and the *Discoglossus* population residing to the south of the Guadalquivir River basin on Iberia is described as a new species. An evolutionary scenario for Iberian and Moroccan populations, consistent with the biochemical and morphological data, is inferred from the physiogeographic history of the region.

INTRODUCTION

Until recently only two species of *Discoglossus* were thought to inhabit Europe. Spanish, French, and Sicilian populations were called *D. pictus*, and populations inhabiting Corsica, Sardinia, Elba, and Monte Argentario, Italy, were called *D. sardus* (Knoepffler, 1961a, 1961b, 1962). Recent electrophoretic examinations of *Discoglossus* have, how-

¹ Address: Fellow in Herpetology, California Academy of Sciences, Golden Gate Park, San Francisco, California 94118.

² Present address: Department of Genetics and Development, University of Illinois, Urbana, Illinois 61801.

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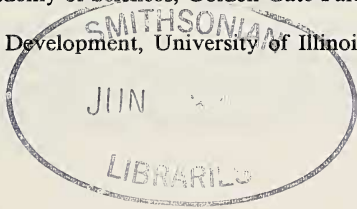




Fig. 1.—Localities of examined specimens of *Discoglossus galganoi* (squares), *D. pictus* (triangles), and *D. jeanneae* (circle). Dotted line traces the presumed northern limit of *D. jeanneae*; see Discussion for further explanation.

ever, revealed two additional species, *D. montalentii* from Corsica (Lanza et al., 1984) and *D. galganoi* from north of the Guadalquivir River basin in Spain and Portugal (Capula et al., 1985).

My interest in *Discoglossus* was initially focused on the amount of genetic differentiation that accumulated between European and African populations after the formation, 5–7 million years ago, of the Strait of Gibraltar (Busack, 1986). Because my electrophoretic study revealed substantial genetic differentiation between Spanish and Moroccan samples, the morphologies of these populations were also compared. Biochemical and morphological data indicate that Iberian populations to the north and to the south of the Guadalquivir River basin are not conspecific, and that neither is conspecific with populations inhabiting northern Morocco. The data supporting this conclusion are presented in the following pages, together with a description of a second species from Spain, and a hypothetical reconstruction of the evolutionary history of Iberian and Moroccan forms of *Discoglossus*.

Table 1.—*Protein systems examined by electrophoresis; enzymes are arranged by Enzyme Commission number.*

Protein (abbreviation)	Enzyme Commission number	Electrophoretic conditions
Albumin (Ab)	—	B
(Oxidoreductases)		
Alcohol dehydrogenase (Adh)	1.1.1.1	A
Glycerol-3-phosphate dehydrogenase (Gpd)	1.1.1.8	D
L-Lactate dehydrogenase (Ldh)	1.1.1.27	F
Malate dehydrogenase (Mdh)	1.1.1.37	F
Malate dehydrogenase (Me)	1.1.1.40	F
Isocitrate dehydrogenase (Icd)	1.1.1.42	E
Phosphogluconate dehydrogenase (Pgd)	1.1.1.44	E
Glutamate dehydrogenase (Glud)	1.4.1.3	D
Superoxide dismutase (Sod)	1.15.1.1	D
(Transferases)		
Aspartate aminotransferase (Aat)	2.6.1.1	D
Hexokinase (Hk)	2.7.1.1	G
Creatine kinase (Ck)	2.7.3.2	G
(Hydrolases)		
Esterase (Est)	3.1.1.1	B
Esterase-D (Est-D)	3.1.1.1	B
Acid phosphatase (Acp)	3.1.3.2	G
Fructose-bisphosphatase (Hdp)	3.1.3.11	D
N-Acetyl-Beta-glucosaminidase (Hex)	3.2.1.30	G
Dipeptidase I, L-Leucyl-L-Alanine (La)	3.4.11	B
Dipeptidase III, L-Leucylglycyl-glycine (Lgg)	3.4.11	C
Dipeptidase IV, L-Phenylalanyl-L-Proline (Pap)	3.4.11	B
Adenosine deaminase (Ada)	3.5.4.4	A
(Lyases)		
Fructose-bisphosphate-aldolase (Ald)	4.1.2.13	H
Aconitate hydratase (Acon)	4.2.1.3	E
(Isomerases)		
Mannose-6-phosphate isomerase (Mpi)	5.3.1.8	E
Glucose-6-phosphate isomerase (Gpi)	5.3.1.9	F
Phosphoglucomutase (Pgm)	5.4.2.2	E

A = Histidine, pH 7.8 gel and electrode buffer (Harris and Hopkinson, 1976), 150v/3h.

B = LiOH A + B, pH 8.2 gel and LiOH A, pH 8.1 electrode buffer (Selander et al., 1971), 300v/3h.

C = Poulik, pH 8.7 gel and borate, pH 8.2 electrode buffer (Selander et al., 1971), 250v/3h.

D = Tris citrate II, pH 8.0 gel and electrode buffer (Selander et al., 1971), 130v/4h.

E = Tris citrate II, pH 8.0 + NADP gel and tris citrate II, pH 8.0 electrode buffer (Selander et al., 1971), 130v/4h.

F = Tris citrate III, pH 7.0 gel and electrode buffer (Ayala et al., 1972), 180v/3h.

G = Tris citrate III, pH 7.0 + 15% glycerine gel and tris citrate III, pH 7.0 electrode buffer (Ayala et al., 1972), 180v/3h.

H = Tris citrate III, pH 7.0 + NAD + 2-mercaptoethanol gel and tris citrate III, pH 7.0 electrode buffer (Ayala et al., 1972), 180v/3h.

MATERIALS AND METHODS

Electrophoresis.—One individual was collected from north of the Guadalquivir River basin in Ávila Province, Spain, at San Martín del Pimpollar (40°22'N, 5°03'W; Fig. 1, square C). Seven specimens were collected south of the Guadalquivir River basin in Cádiz Province, Spain (Fig. 1, circle), near the towns of Facinas (36°08'N, 5°42'W, 5 specimens) and Benalup de Sidonia (36°20'N, 5°49'W, 2 specimens). Specimens collected in Tétouan Prefecture at Chechaouene (35°10'N, 5°16'W; Fig. 1, open triangle; 5 specimens) and in the vicinity of Tleta Tahremt (35°47'N, 5°28'W; Fig. 1, closed triangle; 5 specimens) represent northern Moroccan populations.

Specimens were sacrificed in the field and samples of heart and liver were removed, frozen, and stored in liquid nitrogen (−196°C). In the laboratory, tissues were transferred to a freezer (−76°C) until used in electrophoresis two to 12 months later. Tissue samples were pooled for each animal. Proteins were separated electrophoretically in horizontal starch gels (11.5% hydrolyzed starch, Sigma Chemical Co.) and localized by standard histochemical staining procedures (Ayala et al., 1972; Harris and Hopkinson, 1976; Selander et al., 1971; Table 1). Genetic interpretations of allozymic data were based on criteria developed by Selander et al. (1971). Multiple loci within a protein system were numbered with "1" designating the most anodally migrating set of allelic products. Alleles of a locus were lettered, with "a" representing the most anodally migrating product. Data resulting from the electrophoretic analysis are summarized in Table 2.

Two methods were used to analyze genetic relationships among populations of *Discoglossus*. The first distinguishes patterns of allele distribution among populations that occur as a result of chance association from those that occur too frequently to be chance phenomena. If all patterns of allele distribution are equally probable, the probability, P , that a particular pattern will occur is $1/S_n$ where S_n is the sum of the number of possible patterns of allele distribution. In the case of the three populations of *Discoglossus* being compared, $S_n = 7$ for three items combined three at a time, two at a time, and one at a time. The probability of observing a specific pattern of allele distribution, r , two or more times is given by summing the terms of the binomial expansion:

$$b(r) = \binom{m}{r} P^r (1 - P)^{m-r},$$

where r is the number of replications seen for a given pattern of allele distribution, m is the total number of alleles in the data set, and $\binom{m}{r}$ is the number of possible combinations of m alleles taken r at a time (Straney, 1980; Patton and Smith, 1981).

The second method is the computation of estimates of, and standard errors for, the unbiased minimum genetic distance (\hat{D} ; Nei, 1978, 1971, respectively) between *Discoglossus* populations. Allele frequency data (Table 2) were used directly for the computation of genetic distances and their standard errors.

Morphology.—Capula et al. (1985:tables 2, 4) published comprehensive tables of morphological measurements for *D. galganoi*. I have used the data in these tables to elucidate the morphological features of *D. galganoi* and to make direct assessments of morphological differentiation between *D. galganoi* and other taxa.

Spanish specimens I personally examined were representative of the same Cádiz Province populations as my electrophoretic samples, but collected earlier (between 1969 and 1972). Moroccan specimens included those used for electrophoresis as well as additional material from the same sites. Straight-line measurements of snout–urostyle (SUL), snout (anterior corner of the eye to the tip of the snout), head (posterior angle of the jaw to the tip of the snout), eye (horizontal diameter from posterior corner to anterior corner), tibia, femur, hand (proximal aspect of the central metacarpal tubercle to the tip of the third digit), and foot (proximal aspect of the metatarsal tubercle to the tip of the third digit) lengths were taken to 0.1 mm with dial calipers. Head width (angle of jaws),

interorbital (between the anterior corner of the eyes), and internarial (center to center) distances were also recorded.

Frogs are sometimes sexually dimorphic in many characters and sexes were analyzed separately. The effect of having limited numbers of individuals of each size representing each sex of each population was minimized by converting each character measurement to natural logarithms; the variance of ln-transformed data estimates intrinsic variability and is unaffected by size (Lewontin, 1966; Moriarty, 1977). Transformed data representing each character were subjected to covariance analysis in which snout-urostyle length was selected as the independent variable. Although allometry is correctly assessed only from the study of growth of an individual, I used individuals of different sizes from a population to obtain estimates of allometric coefficients. Identification of dissimilarities in the allometric growth influence by using transformed data is acceptable for comparing populations (Thorpe, 1976).

Linear regression analysis, in which the measurement data were left untransformed, was then performed for variables demonstrating significant differences in allometric growth. For ease of presentation and interpretation, only the resulting slope and intercept values are reported in Table 4. Significance levels for all statistical tests were set (*a priori*) at 0.05 and probabilities are those for committing a Type I error in a two-tailed test.

RESULTS

Biochemical Comparisons

Aat1, Aat2, Acp2, Ada, Est-D, Hdp, Me, Pgm, and Sod were monomorphically expressed among all three populations I examined. Table 2 summarizes the distribution of allozymes at the 25 polymorphic loci I was able to score unambiguously.

Populations of *Discoglossus* residing to the north and to the south of the Guadalquivir River basin in Spain and those inhabiting northern Morocco are genetically well differentiated. Fifteen of 87 alleles identified among these *Discoglossus* samples are shared among all populations. Spanish *Discoglossus* residing north of the Guadalquivir River basin share 17 alleles with individuals from northern Morocco and 23 alleles with *Discoglossus* residing south of the Guadalquivir River basin in Spain. Populations inhabiting the area south of the Guadalquivir River basin share 29 alleles with *Discoglossus* inhabiting northern Morocco (Table 3).

Seventy alleles, however, differentiate between individuals of *Discoglossus* from north of the Guadalquivir River basin in Spain and those from northern Morocco, 64 alleles differentiate individuals from north of the Guadalquivir River basin and those from south of the Guadalquivir River basin in Spain, and 58 alleles differentiate individuals living to the south of the Guadalquivir River basin in Spain from those inhabiting northern Morocco (Tables 2 and 3). The distribution of these alleles contributes to a genetic distance (\bar{D}) of 0.74 ± 0.18 between the Spanish sample from north of the Guadalquivir River basin and the northern Moroccan samples, 0.39 ± 0.12 between Spanish samples residing to the north and to the south of the Guadalquivir

Table 2.—*Genic variation within and among samples of Discoglossus.*

		<i>D. jeanneae</i>	<i>D. galganoi</i>	<i>D. pictus</i>
Number of specimens		7	1	10
Mean heterozygosity per locus		0.16	0.03	0.16
Percentage of loci polymorphic		38.2	2.9	44.1
Locus and alleles				
Ab1	a	0.86	1.00	0.95
	b	0.14	0.00	0.00
	c	0.00	0.00	0.05
Ab2	a	1.00	1.00	0.00
	b	0.00	0.00	1.00
Acon	a	0.57	0.00	1.00
	b	0.29	0.00	0.00
	c	0.14	0.00	0.00
	d	0.00	1.00	0.00
Acp1	a	0.00	0.00	0.10
	b	1.00	1.00	0.90
Adh	a	1.00	1.00	0.80
	b	0.00	0.00	0.20
Ald	a	0.00	1.00	0.00
	b	0.86	0.00	1.00
	c	0.14	0.00	0.00
Ck	a	0.57	0.00	0.10
	b	0.00	0.00	0.10
	c	0.43	0.00	0.75
	d	0.00	0.00	0.05
	e	0.00	1.00	0.00
Est	a	0.00	0.00	0.20
	b	0.00	0.00	0.40
	c	0.29	0.00	0.40
	d	0.14	0.00	0.00
	e	0.57	1.00	0.00
Glud	a	0.00	1.00	0.20
	b	1.00	0.00	0.80
Gpd	a	0.00	1.00	0.00
	b	0.07	0.00	0.00
	c	0.93	0.00	0.50
	d	0.00	0.00	0.50
Gpi	a	1.00	1.00	0.90
	b	0.00	0.00	0.10
Hex	a	0.00	0.00	0.10
	b	1.00	1.00	0.00
	c	0.00	0.00	0.90
Hk1	a	1.00	1.00	0.00
	b	0.00	0.00	1.00
Hk2	a	0.00	1.00	0.00

Table 2.—Continued.

		<i>D. jeanneae</i>	<i>D. galganoi</i>	<i>D. pictus</i>
	b	1.00	0.00	0.00
	c	0.00	0.00	1.00
Icd1	a	0.00	0.00	0.10
	b	0.86	0.00	0.85
	c	0.14	0.00	0.05
	d	0.00	1.00	0.00
Icd2	a	0.00	0.00	1.00
	b	0.00	0.50	0.00
	c	1.00	0.50	0.00
La	a	0.00	0.00	0.10
	b	0.57	1.00	0.90
	c	0.43	0.00	0.00
Ldh1	a	1.00	1.00	0.00
	b	0.00	0.00	1.00
Ldh2	a	1.00	0.00	1.00
	b	0.00	1.00	0.00
Lgg	a	0.00	0.00	1.00
	b	1.00	1.00	0.00
Mdh1	a	0.14	0.00	0.00
	b	0.29	0.00	0.00
	c	0.43	0.00	0.00
	d	0.14	0.00	0.00
	e	0.00	1.00	0.60
	f	0.00	0.00	0.30
	g	0.00	0.00	0.10
Mdh2	a	0.71	0.00	0.90
	b	0.29	0.00	0.10
	c	0.00	1.00	0.00
Mpi	a	0.00	0.00	0.50
	b	0.71	1.00	0.50
	c	0.29	0.00	0.00
Pap	a	0.29	0.00	0.00
	b	0.71	1.00	0.00
	c	0.00	0.00	1.00
Pgd	a	0.86	0.00	0.50
	b	0.00	0.00	0.40
	c	0.14	0.00	0.10
	d	0.00	1.00	0.00

River basin, and 0.39 ± 0.12 between Spanish samples from south of the Guadalquivir River basin and those from northern Morocco.

Morphological Comparisons

Between sexes.—The 19 male and 8 female specimens in the sample of *D. galganoi* examined by Capula et al. (1985) do not demonstrate

Table 3.—Number of replications of the seven possible patterns of allele distribution present among the three populations of *Discoglossus*. Patterns replicated eight times or more do not occur as a result of chance association ($b \leq 0.05$, see *Materials and Methods*) but only alleles shared between two or more populations are informative.

<i>D. galganoi</i>	<i>D. jeanneae</i>	<i>D. pictus</i>	Number
X	X	X	15
	X		14
X			10
		X	24
X		X	2
X	X		8
	X	X	14

sexual dimorphism. There were no significant differences in allometry identified by the covariance analysis.

While no significant difference was found in the distribution of SUL between 14 male and 16 female *Discoglossus* from south of the Guadalquivir River basin in Spain, the allometric relationship between SUL and foot length is significantly different between the sexes of this population ($F = 6.61$, $P < 0.05$). The foot length of a female with an SUL of 45 mm (an intermediate size in the sample of adults of either sex) is approximately 95% the length of the foot of an equivalent-sized male.

Five male and six female *Discoglossus* from northern Morocco demonstrated sexual dimorphism in the allometric relationship between SUL and snout length. While no significant differences were identified between male and female SUL in these samples, the relationship between SUL and snout length is significantly different between sexes ($F = 16.39$, $P \ll 0.05$). At an SUL of 45 mm, the snout length of a female specimen is approximately 86% of the snout length of an equivalent-sized male.

Between populations.—Male specimens of *D. galganoi* differ from male *Discoglossus* from northern Morocco in the allometric growth relationship between SUL and snout ($F = 68.01$, $P \ll 0.05$), head ($F = 63.57$, $P \ll 0.05$), tibia ($F = 10.66$, $P < 0.05$), femur ($F = 16.90$, $P \ll 0.05$) and hand lengths ($F = 55.07$, $P \ll 0.05$) and in the growth relationships of SUL and head width ($F = 5.84$, $P < 0.05$) and SUL and internarial distance ($F = 8.05$, $P < 0.05$). Female specimens representing these populations differ in the allometric relationship between SUL and head ($F = 39.56$, $P \ll 0.05$) and eye ($F = 28.73$, $P \ll 0.05$) lengths.

Male specimens of *D. galganoi* differ from males from south of the Guadalquivir River basin in Spain in the allometric growth relationship

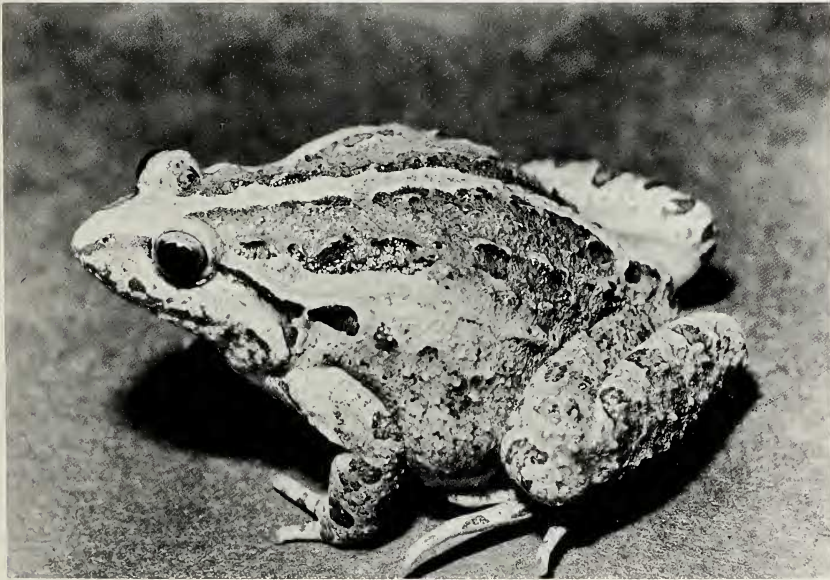
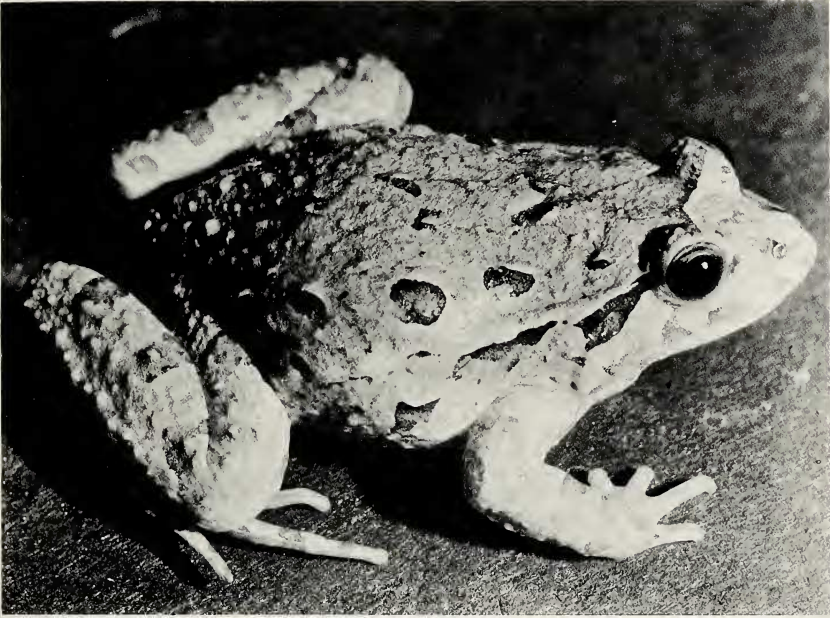


Fig. 2.—Spotted (upper) and striped (lower) color phases of *Discoglossus jeanneae*.

of SUL and snout ($F = 224.33$, $P \ll 0.05$), head ($F = 54.76$, $P \ll 0.05$), tibia ($F = 11.29$, $P \ll 0.05$), femur ($F = 7.08$, $P < 0.05$), and hand lengths ($F = 45.82$, $P \ll 0.05$), and in the allometric relationship between SUL and internarial distance ($F = 5.63$, $P < 0.05$). Female specimens representing these populations differ in the allometry between SUL and head length ($F = 72.34$, $P \ll 0.05$), SUL and hand length ($F = 77.57$, $P \ll 0.05$), and between SUL and internarial distance ($F = 12.62$, $P \ll 0.05$).

Male specimens from south of the Guadalquivir River basin in Spain and those from northern Morocco differ only in the allometric growth relationship between SUL and snout length ($F = 16.47$, $P \ll 0.05$). The allometric growth relationship between SUL and all examined morphological characters is not different in female specimens drawn from these populations.

SYSTEMATIC CONSIDERATIONS

Northern Moroccan populations.—Lanza et al. (1984) demonstrated that specimens of *Discoglossus* from near Barika, Algeria, are genetically very similar to those from Sicily, the type locality of *D. pictus* (Nei's average genetic identity = 0.93, Nei's $D = 0.07$). Although Capula et al. (1985:table 7) do not provide a table of allele frequencies with which I might directly compare results, they do list 12 loci that distinguished Algerian and Tunisian *D. pictus* from Iberian *D. galganoi*. Three of these 12 loci (Ada, Aat1, and Acp2) were found to be monomorphic among populations I compared, and seven were not considered in my study. Only two of these 12 loci (Icd2 and Ldh1) distinguished between Moroccan and Iberian populations in my study. While it is possible that not all Moroccan, Algerian, Tunisian, and Sicilian populations are conspecific, at this time it is zoogeographically and systematically conservative to consider populations of *Discoglossus* inhabiting North Africa and Sicily *D. pictus* Otth, 1837.

Iberian populations residing north of the Guadalquivir Basin.—The albumin immunological distance obtained when specimens from Villaviciosa and Arenas de San Pedro, Spain (Fig. 1A, B, respectively), were compared to those representing *D. pictus* from Tleta Tarhremt, Morocco (Fig. 1, closed triangle), was 17 units (Maxson and Szymura, 1984:249). The unbiased genetic distance (Nei's \hat{D}) between one individual I collected from San Martín del Pimpollar, Spain (Fig. 1C), and *D. pictus* from northern Morocco was 0.74 ± 0.18 . Capula et al. (1985) reported a genetic distance (Nei's D) of 0.58 between their pooled samples representing Portugal and central Spain and those from Algeria and Tunisia.

It is apparent from genetic (Capula et al., 1985:table 9) and mor-

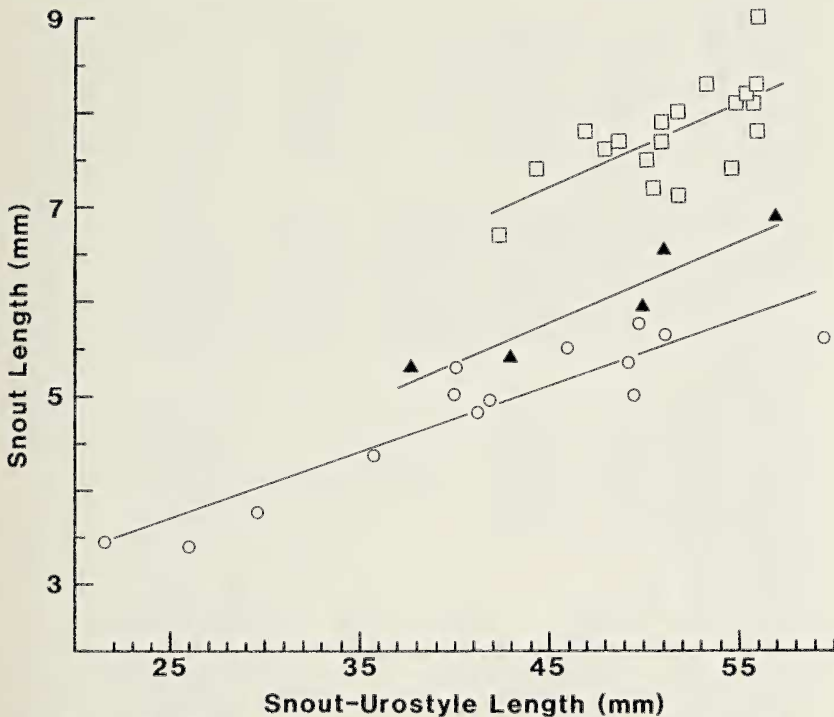


Fig. 3.—Snout length regressed on snout-urostyle length in male *Discoglossus galganoi* (squares), *D. pictus* (triangles), and *D. jeanneae* (circles). See Table 4 for regression coefficients.

phological comparisons (this study, Results) that Iberian populations of *Discoglossus* residing north of the Guadalquivir River basin are clearly differentiated from those inhabiting northern Morocco and are deserving of the species status recently ascribed to them by Capula et al. (1985). Whether or not, however, the specimens Maxson and Szymura (1984) and I examined biochemically (Fig. 1A–C) actually represent *D. galganoi* is unclear. Until data with which to further assess the taxonomic status of Iberian populations residing to the north of the Guadalquivir River basin become available, these populations are best referred to *D. galganoi* Capula et al., 1985.

Iberian populations residing south of the Guadalquivir Basin.—*Discoglossus* from south of the Guadalquivir River basin in Spain are morphologically and genetically different from both Moroccan *D. pictus* and *D. galganoi* (Tables 2, 3, and Results). I consider the extent of these differences to be representative of species level differentiation and designate the new species herewith.

Table 4.—Estimates of slope (b) and intercept (a) obtained when measurements derived from various morphological features (y) were regressed against snout-urostyle length (x) in *Discoglossus galganoi*, *D. jeanneae*, and *D. pictus*. Linear regression results are reported only for those allometric growth characteristics that differed significantly in an analysis of covariance; an analysis of variance technique (Sokal and Rohlf, 1981:471) was used to assess the hypothesis that each linear regression was significant (values of F_s and associated significance levels [P] for this statistical test are provided).

Statistics	<i>D. galganoi</i>		<i>D. jeanneae</i>		<i>D. pictus</i>	
	Males	Females	Males	Females	Males	Females
	<i>Head length</i>					
b ± SE	0.24 ± 0.04	0.20 ± 0.04	0.20 ± 0.02	0.18 ± 0.03	0.16 ± 0.04	0.20 ± 0.06
a ± SE	2.40 ± 2.14	4.51 ± 1.61	2.60 ± 0.65	3.10 ± 1.26	3.77 ± 1.72	2.39 ± 2.34
F_s , P	34.97, <0.05	31.36, <0.05	168.67, <0.05	45.29, <0.05	20.86, <0.05	12.25, =0.05
	<i>Head width</i>					
b ± SE	0.33 ± 0.04	—	—	—	0.23 ± 0.03	—
a ± SE	0.15 ± 1.95	—	—	—	4.30 ± 1.54	—
F_s , P	74.52, <0.05	—	—	—	49.79, <0.05	—
	<i>Snout length</i>					
b ± SE	0.09 ± 0.02	—	0.07 ± 0.01	—	0.09 ± 0.02	—
a ± SE	3.14 ± 1.15	—	1.90 ± 0.35	—	1.86 ± 0.81	—
F_s , P	16.26, <0.05	—	75.05, <0.05	—	26.84, <0.05	—
	<i>Internarial distance</i>					
b ± SE	0.05 ± 0.01	0.04 ± 0.01	0.05 ± 4.41 × 10 ⁻³	0.06 ± 0.01	0.04 ± 0.01	—
a ± SE	0.55 ± 0.59	0.76 ± 0.43	0.36 ± 0.19	0.42 ± 0.44	0.97 ± 0.41	—
F_s , P	17.46, <0.05	20.43, <0.05	158.89, <0.05	36.11, <0.05	26.70, <0.05	—

Table 4.—Continued.

Statistics	<i>D. galganoi</i>		<i>D. jeanneae</i>		<i>D. pictus</i>	
	Males	Females	Males	Females	Males	Females
			<i>Eye length</i>			
b ± SE	—	0.08 ± 0.01	—	—	—	0.07 ± 0.01
a ± SE	—	0.59 ± 0.69	—	—	—	0.15 ± 0.53
F ₅₃ , P	—	27.53, <<0.05	—	—	—	37.86, <0.05
			<i>Tibia length</i>			
b ± SE	0.34 ± 0.07	—	0.40 ± 0.04	—	0.29 ± 0.04	—
a ± SE	9.96 ± 3.58	—	4.88 ± 1.52	—	10.50 ± 1.76	—
F ₅₃ , P	24.26, <<0.05	—	126.55, <<0.05	—	63.62, <0.05	—
			<i>Femur length</i>			
b ± SE	0.29 ± 0.08	—	0.41 ± 0.02	—	0.18 ± 0.04	—
a ± SE	9.65 ± 4.39	—	2.39 ± 0.84	—	12.40 ± 2.00	—
F ₅₃ , P	11.72, <<0.05	—	428.16, <<0.05	—	18.46, <0.05	—
			<i>Hand length</i>			
b ± SE	0.10 ± 0.02	0.10 ± 0.02	0.13 ± 0.01	0.11 ± 0.02	0.10 ± 0.02	—
a ± SE	2.72 ± 1.15	2.72 ± 0.96	-0.09 ± 0.33	0.81 ± 0.71	1.25 ± 0.83	—
F ₅₃ , P	19.80, <<0.05	21.59, <0.05	299.02, <<0.05	51.28, <<0.05	36.64, <0.05	—

Discoglossus jeanneae, new species

(Fig. 2)

Holotype.—Carnegie Museum of Natural History (CM) 54657, an adult female, along highway C-440, 15 km ESE Alcalá de los Gazules, Cádiz Province, Spain, 6 April 1971, Stephen D. Busack.

Paratypes (21, all from Cádiz Province, Spain).—CM 52126, female, along highway C-440, 12.9 km ESE Alcalá de los Gazules, 18 October 1969; CM 52128–52129, two females, along highway C-440, 13.8 km WNW Los Barrios, 18 October 1969; CM 52475–52476, two males, along highway C-440, 6.6 km NW Algeciras, 10 January 1970; CM 53087, male, along highway C-440, between 7.6 and 7.7 km NW Los Barrios, 12 March 1970; CM 53119, male, along highway CA-221, 21.2 km ENE Facinas, 10 April 1970; CM 53324, male, along highway C-440, 9.01 km NW Los Barrios, 18 June 1970; CM 53884a–d, 4 females, along highway C-440, between 4.0 km WNW Casas del Castaño and 5.0 km WNW Los Barrios, 8 October 1970; CM 54244, female, along highway C-440, between 19 km ESE Alcalá de los Gazules and 1 km WNW Casas del Castaño, 18 November 1970; CM 54581, female, and CM 54582, male, along highway C-440, between 3.7 and 11.4 km WNW Los Barrios, 19 March 1971; CM 54608–54610, three females, along highway CA-P-2112, between 12.6 and 14.2 km NNE Benalup de Sidonia, 2 April 1971; CM 54704, male, along highway C-440, 0.3 km WNW Casas del Castaño, 16 May 1971; CM 55742–55743, two males, and CM 55744, female, along highway C-440, between 18.4 and 22.7 km WNW Los Barrios, 15 January 1972.

Diagnosis.—*Discoglossus jeanneae* is similar in coloration and pattern to *D. galganoi* and *D. pictus*, but is distinguished from them by biochemical and morphological characters. *Discoglossus jeanneae* and *D. pictus* share no alleles at six electrophoretic loci (Ab2, Hk1, Hk2, Icd2, Ldh1, and Lgg), *D. jeanneae* and *D. galganoi* share no alleles at three loci (Glud, Hk2, and Ldh2).

Male *D. jeanneae* have a shorter snout than males of either *D. pictus* or *D. galganoi* of similar SUL (Fig. 3, Table 4). Male *D. jeanneae* also have a greater head length, a shorter internarial distance, and shorter tibia, femur, and hand lengths than male *D. galganoi* of comparable SUL (Table 4). Female *D. jeanneae* have a shorter head and hand lengths, and shorter internarial distances, than *D. galganoi* of similar SUL (Table 4).

Description of holotype.—An adult female with the following measurements (mm): SUL 47.9, snout length 5.1, head length 10.8, head width 15.1, horizontal diameter of eye 4.1, interorbital distance 7.5, internarial distance 3.0, tibia length 23.4, femur length 21.7, hand length 5.9, foot length 15.1. Choanae oblong and relatively large; prevomerine dentigerous processes in two nearly straight series, each with 11 teeth, located just behind the choanae and separated by a distance less than half that of the diameter of a choana. Tongue roundish, thick, scarcely free behind. Nostrils dorsal, much closer to the tip of the snout than to the eye, horizontal diameter of the eye slightly greater than the distance from the naris to the eye. Tympanum indistinct. Fingers rel-

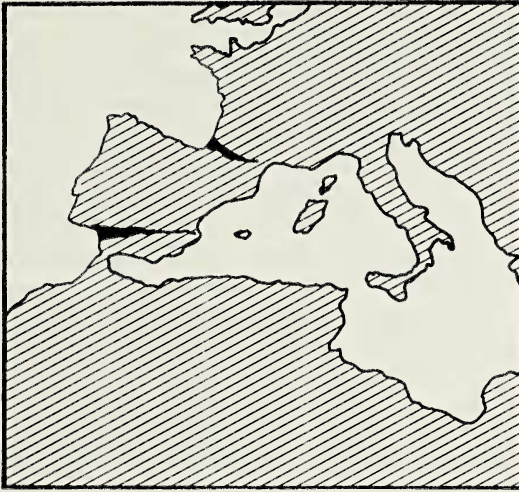


Fig. 4.—A Lower Miocene reconstruction of the Iberian peninsula and North Africa; heavily blackened areas indicate marine incursions (after Illies, 1975).

atively short, unwebbed; III, IV, II, I in order of decreasing length. Three prominent palmar tubercles, the largest at the base of finger IV, the next largest at the base of finger I, and the smallest in the center. Toes slender, very slightly webbed, IV, III, V, II, I in order of decreasing length. No subarticular tubercles, no tarsal fold; small ellipsoidal metatarsal tubercle. Heels overlap slightly when femora are held at right angles to the body axis. Skin of dorsum with several irregularly positioned diminutive, pustules, skin of venter smooth.

Coloration of holotype (in alcohol).—The ground color of the top of the head is citrine drab from mid-eye to snout. Beginning at mid-eye, and continuing to just below the area of front limb insertion, a dark olive patch reminiscent of a large italicized “X” appears on a ground color of a lighter shade of dark olive. A prominent (6.5 mm in length) and elongated teardrop-shaped patch of chaetura drab angles ventro-posteriorly from the posterior corner of the eye. This patch, and the ground color of the “X,” are edged in citrine drab. Small faint patches shaped as triangles oriented with the base down appear along the upper lip; a light stripe extends from the front corner of the eye through the nostril.

Colored as the “X,” a lightly-horned, heart-shaped, shield extends over the central dorsum. The upper half of the shield is edged in citrine drab on a ground color of lighter dark olive; intense asymmetrical patches of dark olive line the lower “V” and the upper and outer curves of this shield. A broken dorsolateral line of citrine drab is present.

In lateral view, an ovoid dark olive blotch appears below the dorso-lateral line and just above the area of insertion of the front limb. The interaxillary region is light deep olive blending into dark olive buff towards the venter; the dark olive buff blends into buffy brown in the region of the groin. The venter is deep olive buff and unpatterned.

The upper surfaces of the hind legs are clove brown with irregular transverse blotches of fuscous black; upper surfaces of the front legs are citrine drab with three deep olive transverse blotches.

Pattern polymorphism (in alcohol).—Spotted individuals are most common, striped specimens are less common (Fig. 2), and occasionally an unpatterned *D. jeanneae* is found. Unstriped individuals exhibit either a complete or broken anteriodorsal "X." All have a teardrop eye patch, triangular blotches on the upper labium, and, in varying degrees of distinction, a stripe extending from the front corner of the eye through the nostril. A broken dorsolateral line of citrine drab also appears, at times faint, at times pronounced, in all unstriped individuals.

Unstriped individuals have a ground color which varies from citrine drab to light dark olive to fuscous. Coloration on the posterior portion of the dorsum varies from a pattern in which prominent spots are connected to suggest a shield-like shape to a simple pattern of widely spaced and barely discernable spots. Ventral coloration is generally unpatterned deep olive buff; some specimens, however, demonstrate olive or clove brown pigmentation of varying intensity on the lower jaw and upper pectoral region.

In the striped paratype (CM 53119), the "X" and shield symmetry are broken by a medial stripe of citrine drab. The resultant halves of the "X" and shield merge on either side of the medial stripe to form two solid regions of dark olive, one on the right, one on the left, and each between the medial stripe and the dorsolateral region. The small ovoid blotch found below the dorsolateral ridge and above the area of limb insertion in spotted individuals is continuous with the teardrop eye patch and forms a single blotch in this specimen. The eye-nostril stripes and upper labial triangles are pronounced and, along the dorsolateral region, there is a prominent citrine drab stripe extending from the eye to the groin. The outer edge of this stripe exhibits the darker coloration reminiscent of the dark olive upper and outer curves of the heart-shaped shield described for the holotype.

Etymology.—Jeanne A. Visnaw accompanied me during four months of field work in Spain and Morocco during 1982; in spite of what she learned during her first trip abroad, Jeanne again accompanied me in 1983. While the husbands and wives of graduate students often contribute substantially to the success of their spouses, few routinely sacrifice as much or give as unselfishly as my wife has. I dedicate this new Spanish frog to her.

DISCUSSION

Physiogeographic changes have been extensive in this area of the western Mediterranean region in the last few million years. The Betic Cordillera (an alpine ridge located on the Spanish Meseta between Cádiz in the west and Alicante in the east) was subjected to folding and extensively restructured during Miocene-Pliocene. Lower Miocene Atlantic waters flowed to the Mediterranean through what is now the Guadalquivir River basin (Figs. 4–5; Berggren and Van Couvering, 1974; Illies, 1975; Le Pichon et al., 1972), Neogene sedimentation filled the younger, western portion (Córdoba-Sevilla) of the Guadalquivir Basin and the northern portion began to emerge from considerable depth at the end of the Miocene (Tjalsma, 1971:120–125), and Pliocene events allowed Atlantic waters to form the Strait of Gibraltar (Fernix et al., 1967; Hsü, 1983; and Mantura, 1977).

Geologic events such as these have a direct effect on sexually reproducing organisms. The rate at which genetic divergence accumulates between populations is believed to be a function of the time those populations have been separated (Zuckerlandl and Pauling, 1965). If this is true, the more genetically differentiated two populations of terrestrial anurans are, the longer they have been separated. Of the three patterns of allele distribution that are phylogenetically informative among these three populations of *Discoglossus*, *D. galganoi* and Moroccan *D. pictus* share only two, *D. galganoi* and *D. jeanneae* share 8, and *D. jeanneae* and Moroccan *D. pictus* share 14 (Table 3). If unbiased genetic distance units (\bar{D}) are considered, Moroccan *D. pictus* are separated from *D. galganoi* by 0.74 ± 0.18 units and *D. jeanneae* is separated from both *D. galganoi* and Moroccan *D. pictus* by 0.39 ± 0.12 units (Fig. 5). *Discoglossus galganoi* and Moroccan *D. pictus* are probably not sister species.

Males of *D. galganoi* are more morphologically differentiated from males of *D. jeanneae* and males of *D. pictus* than are female *D. galganoi* from female *D. jeanneae* or *D. pictus*. The allometric growth relationship between SUL and 10 morphological characters were compared among males and females of these three species and male *D. galganoi* differ from Moroccan *D. pictus* in seven such relationships, females differ in only one. Male *D. galganoi* differ from *D. jeanneae* in six allometric relationships, females differ in three. Male *D. jeanneae* appear to be little changed from Moroccan *D. pictus*, however, as only one allometric relationship is clearly different; female *D. jeanneae*, on the other hand, demonstrate no differences among any of the 10 allometric growth relationships when compared with female *D. pictus*.

The fossil record of *Discoglossus* is limited. Middle Miocene remains from Beni-Mellal, Morocco, referred to the genus *Discoglossus* by Vernaud-Grazzini (1966), have been reassigned to the extinct discoglossid

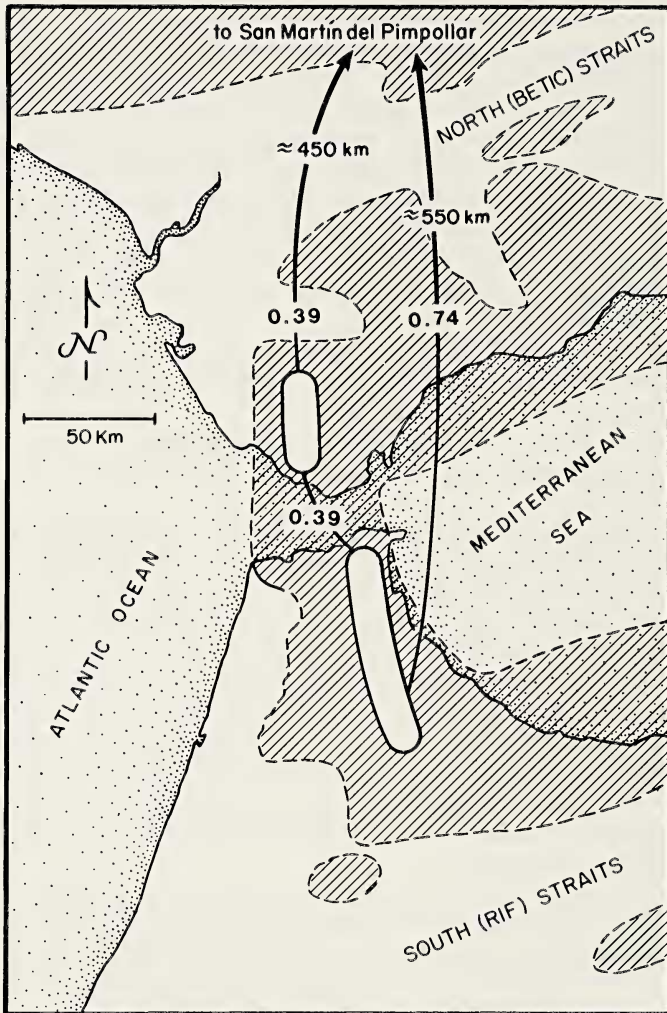


Fig. 5.—A mid-Miocene reconstruction of the Gibraltar area (after Fernix et al., 1967). Shaded areas represent emergent land; geographic and unbiased genetic distances between sampled populations of *Discoglossus* are indicated.

genus *Latonia* by Sanchiz and Alcover (*in litt.*); as a result, fossil representatives of *Discoglossus* are unknown in North Africa. The Lower Miocene *Discoglossus troschelii* from Rott, Germany, was considered conspecific with *D. pictus* (= ? *galganoi*) from Spain (Boulenger, 1891). Neogene specimens from Escobosa de Calatañazor (Soria), Venta del Moro (Valencia), and Alcoy (Alicante), Pliocene samples from El Ar-

quillo II (Teruel), and mid-Pleistocene specimens from Arganda (Madrid), attest to the age, persistence without morphological change, and widespread nature of *D. pictus* (= ? *galganoi*) throughout prehistoric Iberia (Sanchiz, 1977a, 1977b).

The electrophoretic and morphological data coincide well with one evolutionary scenario that may be inferred from the geographic history of the region. The ancestral stock of *D. galganoi*, which once populated the Spanish Meseta, and that of *D. pictus*-*D. jeanneae*, which once populated an area now known as southern Spain and northern Morocco, suffered temporal and climatic separation dating from the Lower Miocene. The Pliocene formation of the Strait of Gibraltar then divided ancestral *D. pictus*-*D. jeanneae* populations into two populations which evolved to become *D. jeanneae* and *D. pictus*.

Additional research is clearly necessary before we can fully understand the evolutionary history, distributional limits, and taxonomy of Iberian and North African *Discoglossus*. Until additional data becomes available, however, conservative limits for the distribution of *D. jeanneae* may be drawn. These would include the northern edge of the Guadalquivir River basin (Fig. 1, dotted line), regions inundated during Miocene flooding that currently lay to the east of the headwaters of the Guadalquivir River (Fig. 1, dotted line with question marks), and the shores of the Atlantic Ocean and the Strait of Gibraltar.

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SPECIMENS EXAMINED

D. jeanneae.—(Electrophoretic Analysis): SDB 1556, 1691, 1905, 1906, 1930, 1949, 1954, and 1989 at the Universidad de León, Spain. (Morphological Analysis): Carnegie Museum of Natural History (CM) 52125–52129, 52475–52477, 52537, 52626, 53087–53088, 53119–53120, 53324, 53884 (4 specimens), 54244, 54581–54582, 54608–54610, 54657, 54704, and 55742–55743.

D. galganoi.—(Electrophoretic Analysis): SDB 1691 at the Universidad de León.

D. pictus.—(Electrophoretic Analysis): SDB 1773 (2 specimens) and SDB 1774 (3 specimens) at the Museum of Vertebrate Zoology, University of California, Berkeley (MVZ); MVZ 186124–186125 and 186132–186134. (Morphological Analysis): MVZ 186124–186134.

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